REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

In the October 20, 2004 Office Action, the Examiner has maintained the two previous art rejections citing, respectively, Hong et al. (U.S. Patent 5,834,253) ("the '253 patent") and Walker (U.S. Patent 5,7612,124) in view of the '253 patent.

Specifically, claims 9-11 and 18-35 are rejected under 35 U.S.C. §102(b) as anticipated by the '253 patent. As noted in our last response, the '253 patent teaches using a *Bacillus stearothermophilus* DNA polymerase with proof-reading 3'-5' exonuclease activity to perform a **single** enzymatic DNA primer extension for the purpose of ".....sequencing of a DNA strand from a template....". In this patent, there is no description or even suggestion of cycling fluctuations of temperature in the enzymatic reaction mixture for repeated cycle extension of DNA in the practice. For someone of ordinary skill in this art following the teaching of the '253 patent, the newly extended DNA fragments generated in the test tube would not and could not exceed the templates in copy number at the end of the reaction, because the DNA templates cannot be used more than once.

In response, on pages 3-4 of the office action the Examiner contended that independent claims 9 and 30 as currently pending recite that in step (ii) the cycle primer extension reaction occurs for "a sufficient number of times"—which arguably includes a single cycle, which arguably is taught by the '253 patent. To clarify the matter, we have amended all independent claims 9, 22 and 30 to definitively read that the cycle primer extension reaction is repeated at least two times and until the sequencing primer molecules are extended to desired lengths. Thus, for this reason alone it is believed that our claims are clearly distinguishable over the '253 patent.

The Examiner has also stated that the '253 patent does in fact teach the use of about 10% glycerol in the reaction mixture which inherently teaches its use as a melting agent—contrary to applicant's previous argument that the '253 patent teaches only the

use of 50% glycerol as a preservative. The Examiner cited column 19, lines 6-11 as support.

However, in context the disclosure at column 19, lines 6-11, actually referred to the use of 50% glycerol as a preservative of Bst DNA polymerase. In this setting, the 50% glycerol was diluted to a negligible concentration in the final enzymatic mixture—so negligible that it could not possibly function as a melting agent, even if this were in the minds of the authors. Reading from column 18, line 64 onward, one can calculate the actual formulation, and summarize the ingredients with its glycerol preservative concentration in the final reaction mixture as follows:

DNA sequencing primer	1.0 µl
ssDNA template	7.0 µl
Buffer	2.0 µl
35S dATP	1.5 µl
Bst DNA polymerase in 50% glycerol	1.0 µl
Total volume	12.5 µl

At this point the concentration of glycerol in the above mixture is 50% /12.5 = 4%.

In order to conduct DNA polymerization reaction, an aliquot of 2.5 µl is pipetted out of the above mixture and added to a tube containing 2.0 µl of the 4 dNTPs plus one ddNTP as terminator in the enzymatic chain extension/termination classic Sanger reaction. There the final concentration of glycerol in the reaction mixture is 4% / (2.5 + 2) = 4%/4.5 = 0.89%. Therefore, in the '253 patent, glycerol was only used as a preservative of an enzyme, and its final concentration in the reaction was less that 1%--NOT 10% and therefore NOT as a melting agent to cause denaturation of the double-stranded DNA for repeated cycle primer extension. In contrast, in the instant invention, the claim of glycerol is in the range of 10-20%--at least 10 times the concentration contributed by the glycerol used as preservative in the cited example in the '253 patent.

The point here is that the '253 patent only discloses a method for single DNA primer extension—there is no suggestion whatsoever of any methods for repeated or cycle primer extension, which is required in all of our claims.

Claims 1-8 are rejected under 35 U.S.C. §103(a) as obvious over Walker (U.S. Patent 5,7612,124) ("the '124 patent") in view of Hong et al. (U.S. Patent No. 5,834,253). Like the previous rejection discussed above, the Examiner is arguing that independent claim 1 does not require repeated low temperature cycle extension. We have amended claim 1 to definitively recite that the method includes the step of "repeating the cycle primer extension reaction at least two times and until the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product of a desired length." Thus, for this reason alone it is believed that our claims 1-8 are clearly distinguishable over the '253 patent alone or in combination with the '124 patent.

In the office action, the Examiner has argued that the '253 patent DNA polymerase is useable at low temperatures. We respectfully note that this is a misunderstanding of the '253 patent as it would relate to our claimed invention. We cannot disagree that the '253 patent DNA polymerase is useable at low temperature—this is true for many other DNA polymerase. But these DNA polymerases cannot be used for low temperature cycle extension for more than one time without the correct concentration of glycerol as required in our claims. This is borne out by the disclosures of both the '253 patent and the '124 patent, since the '253 patent only discloses a method for single DNA primer extension, and the '124 patent teaches amplification of nucleic acids at a single temperature (that is, without cycling).

Given the serious deficiencies in each of the '253 patent and the '124 patent, we submit that it is unreasonable to assume that a fair reading of both these references would have lead someone having ordinary skill in this art to our invention. Someone having ordinary skill in this art would not have been able to combine the single-extension methods of these two patents to design our claimed methods using repeated low temperature cycle extension of DNA for amplification. It simply would not have been obvious to combine these references to achieve our claimed invention.

Withdrawal of this rejection is therefore respectfully requested.

All of the Examiner's outstanding rejections and objections have been addressed, and the application is believed to be in allowable form. Notice to that effect is earnestly

solicited. No amendment made was related to the statutory requirements of patentability unless expressly stated herein, and no amendment made was for the purpose of narrowing the scope of any claim unless we argued above that such amendment was made to distinguish over a particular reference or combination of references.

If the Examiner has any questions or would like to make suggestions as to claim language, the Examiner is encouraged to contact Marlana K. Titus at (301) 977-7227.

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